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(21) International Application Number: <b>PCT/EP98/04223</b> (22) International Filing Date: <b>7 July 1998 (07.07.98)</b> (30) Priority Data: <b>08/900,795</b> <b>25 July 1997 (25.07.97)</b> <b>US</b> (71) Applicant (for AU BB CA CY GB GH GM IE IL KE LC LK LS MN MW NZ SD SG SL SZ TT UG ZW only): <b>UNILEVER PLC [GB/GB]; Unilever, Blackfriars, London EC4P 4BQ (GB).</b> (71) Applicant (for all designated States except AU BB CA CY GB GH GM IE IL KE LC LK LS MN MW NZ SD SG SL SZ TT UG ZW): <b>UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).</b> (72) Inventors: <b>CARSON, Robert, George; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). PATEL, Krupa; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). CARLOMUSTO, Marieann; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). BOSKO, Carol, Annette; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). PILLAI, Sreekumar; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US).</b>		(74) Agent: <b>ROTS, M., J., F.; Unilever plc, Patent Division, Colworth House, Sharnbrook, Bedford MK44 1LQ (GB).</b> (81) Designated States: <b>AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b> <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: <b>COSMETIC COMPOSITIONS</b> (57) Abstract <p>Resveratrol, a component of a variety of common edible plants, including peanuts and red grapes, is a phytoestrogen. Resveratrol inhibits proliferation of skin epidermal cells (keratinocytes) and stimulates their differentiation. Resveratrol was also found to alleviate skin irritation that may be caused by alpha-hydroxy acids. Resveratrol is useful in improving the appearance of wrinkled, lined, dry, flaky, aged or photodamaged skin and improving skin thickness, elasticity, flexibility, radiance, glow and plumpness.</p>		

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COSMETIC COMPOSITIONS

## FIELD OF THE INVENTION

5           Cosmetic compositions containing resveratrol, a natural estrogen derived from plants, and cosmetic methods of conditioning skin by applying such compositions to the skin.

## BACKGROUND OF THE INVENTION

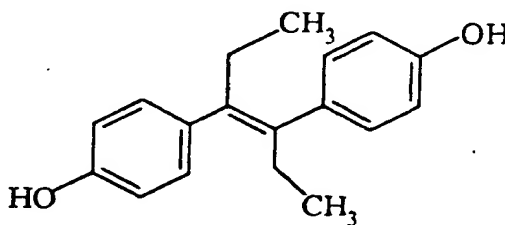
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          The human skin consists of two major layers, the bottom thicker layer, dermis and the top thinner layer the epidermis. Dermis is the layer which provides the strength, elasticity and the thickness to the skin. With aging, the thickness of the dermal layer is reduced and this is  
15           believed to be partially responsible for the formation of wrinkles in aging skin. The top layer of human skin or the epidermis which provides the resilience and the barrier properties of the skin, is composed of many different cell  
20           types. Keratinocytes are the major cell type of the epidermis (75-80% of the total number of cells in the human epidermis). Within the epidermis the keratinocytes reside in four distinct stages of differentiation. Epidermal  
25           differentiation is important for providing the essential function of the skin, namely to provide a protective barrier against the outside environment and to prevent loss of water from the body. Formation of the cornified envelope is the final stage of keratinocyte differentiation. The enzyme  
30           responsible for the formation of cornified envelopes, transglutaminase is a marker of epidermal differentiation. Agents which increase the thickness of the dermal layer and increase the differentiation of keratinocytes in the epidermal layer should therefore be ideal compounds for providing skin conditioning and anti-aging benefits.

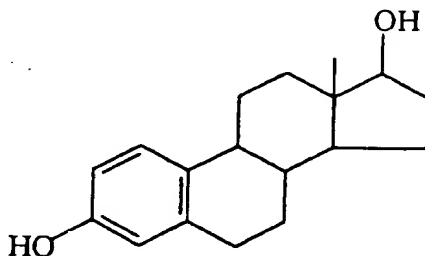
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Estrogens and synthetic compounds which act like estrogens are known to increase the thickness of the dermal layer and reduce wrinkle formation in the aging skin. Changes in the skin such as skin dryness, loss of skin elasticity and plumpness occurring after menopause are attributed to the lack of estrogen production. Estrogen therapy prevents or slows down many of the changes associated with aging skin (Creidi et al., Effect of a conjugated oestrogen cream (Premarin®) on aging facial skin, *Maturitas*, 19, p.211-23, 1994). A synthetic estrogen, diethyl stilbestrol, has the following structure:



This structure is very different from the structure of natural estrogen, estradiol:



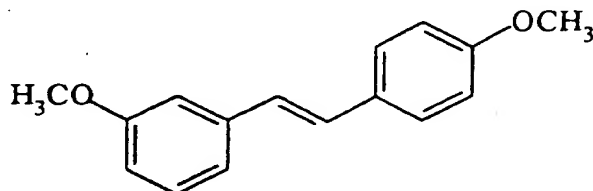
In recent years, phytoestrogens (i.e., natural compounds which have estrogen-like activity and which are found in plants) have been increasingly used for therapeutic purposes. Some of the uses described are as hypocholesterolemic and antiatherogenic agents, treatment of

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cardiovascular diseases especially in postmenopausal women, treatment for osteoporosis in the elderly and as an anticancer agent especially against breast cancer, endometrial and cervical cancer in women (Knight et al.,  
5 Phytoestrogens - a short review, Maturitas, 22: 167-75, 1995).

The consumer demand for "natural" based products has been growing in recent years. The consumers perceive chemical  
10 synthesis as environmentally unsafe. A chemically synthesized ingredient may contain harsh chemicals. Natural products are perceived as pure and mild and superior to chemically synthesized products. However, delivering a cosmetic benefit from plant sources is not trivial. In order to derive a real  
15 benefit from a "natural" source a specific active in the plant has to be identified which truly delivers a cosmetic benefit.

One known phytoestrogen is photoanethole:



20 Photoanethole has not been described for topical or cosmetic use.

25

The present invention is based at least in part on the discoveries that resveratrol is a phytoestrogen, that it inhibits keratinocyte proliferation, increases keratinocyte differentiation, and alleviates irritation or sting  
30 potentially associated with the use of alpha-hydroxy acids.

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Resveratrol is a compound found in a variety of plants. Isolation and characterization of resveratrol have been described from a variety of plants such as the roots of Japanese knotweed (Powell et al., Phytochemistry 35, p.335, 1994), from wine and grapes (Goldberg et al; J. Agric. Food Chem., 43, p.1820, 1995 and Cellotti et al., "Resveratrol content of some wines obtained from dried Valpolicella grapes: Recioto and Amarone., J chromatogr A (Netherlands) 730: 47-52,1996), and from peanut plant cultures (Kindl et al., US patent 5391724). Red grapes and red wine contain high amounts of resveratrol and this compound is claimed as one of the reasons for cardiovascular health in wine drinkers. In addition, resveratrol has been shown to be a potent cancer chemopreventive agent and an anti-inflammatory agent. Resveratrol has also been reported to induce differentiation of human promyelocytic leukemia cells (Jang et al., Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275: 218-220, 1997). Jang et al describe resveratrol's use as an anticancer agent against carcinogen-treated mouse skin cells in culture.

Cosmetic compositions containing grape extract have been described. See for instance abstract of Japanese patent application 06336421 ("JP '421"), disclosing the use of 0.5% grape extract in cosmetic compositions. Scafildi et al. (US Patent 5,683,683) and Zabotto et al. (US Patent 5,439,672) disclose cosmetic compositions containing grape seed oil. Griat et al. (US Patent 5,171,577) disclose cosmetic foams containing cosmetic pips. None of these disclosures, except JP '421, mentions any amount of the grape to be used. JP '421 teaches the presence of 0.5% of grape extract. According to Agricultural Research Service of the United States Department of Agriculture, resveratrol concentration in whole berries is about 15 ppm. Then, the

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resveratrol concentration in 0.5% grape seed extract is 0.33 micromolar or 0.0000075 wt.%.

The art discussed above does not describe the use of resveratrol for skin care or cosmetic use, does not teach that resveratrol is a phytoestrogen, or that it inhibits keratinocyte proliferation, or that it promotes differentiation of keratinocytes, or that it controls skin irritation caused by alpha-hydroxy acids.

#### SUMMARY OF THE INVENTION

The present invention includes skin care composition comprising resveratrol in an amount of from 0.00002 to 10 wt.% and a cosmetically acceptable vehicle.

The present invention also includes a method of improving or preventing the condition of wrinkled, lined, dry, flaky, aged or photodamaged skin and improving skin thickness, elasticity, flexibility, radiance, glow and plumpness, which method includes applying to the skin the inventive composition. Compositions of the invention are intended for topical application to mammalian skin which is already dry, flaky, lined, wrinkled, aged, photodamaged, or the inventive compositions may be applied prophylactically to normal healthy skin to prevent or reduce the deteriorative changes.

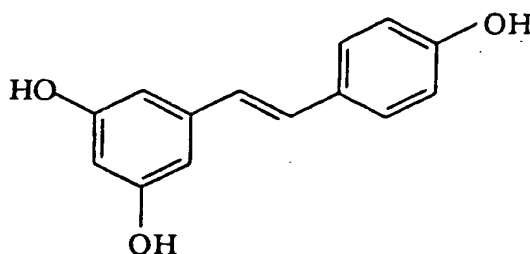
The present invention also includes cosmetic methods of delivering estrogenic activity to the skin, inhibiting keratinocyte proliferation in human skin and increasing keratinocyte differentiation. The invention further includes a cosmetic method of controlling skin irritation, sting or inflammation which may be caused by alpha-hydroxy acids. In this respect, the invention also includes

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cosmetic composition containing resveratrol in combination with an alpha-hydroxy acid.

#### DETAILED DESCRIPTION OF THE INVENTION

Resveratrol (also known as 5-parahydroxystyryl resorcinol, or 3,4',5-stilbenetriol) is an essential ingredient of the inventive composition. Resveratrol has the following structure:



Resveratrol may be obtained commercially from Sigma.

In general, the amount of resveratrol in the inventive compositions is in the range of from 0.00002 to 10 % by weight composition. Preferably in order to lower cost and maximize the effect the amount of resveratrol is in the range of from 0.001% to 5% and most preferably is in the range of from 0.1% to 5%.

The composition according to the invention also comprises a cosmetically acceptable vehicle to act as a diluant, dispersant or carrier for resveratrol in the composition, so as to facilitate its distribution when the composition is applied to the skin.



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Vehicles other than or in addition to water can include liquid or solid emollients, solvents, humectants, thickeners and powders. An especially preferred nonaqueous carrier is a polydimethyl siloxane and/or a polydimethyl phenyl siloxane. Silicones of this invention may be those with viscosities ranging anywhere from about 10 to 10,000,000mm<sup>2</sup>/s (centistokes) at 25°C. Especially desirable are mixtures of low and high viscosity silicones. These silicones are available from the General Electric Company under trademarks Vicasil, SE and SF and from the Dow Corning Company under the 200 and 550 Series. Amounts of silicone which can be utilized in the compositions of this invention range anywhere from 5% to 95%, preferably from 25% to 90% by weight of the composition.

The cosmetically acceptable vehicle will usually form from 5% to 99.9%, preferably from 25% to 80% by weight of the composition, and can, in the absence of other cosmetic adjuncts, form the balance of the composition. Preferably, the vehicle is at least 80 wt.% water, by weight of the vehicle. Preferably, water comprises at least 50 wt.% of the inventive composition, most preferably from 60 to 80 wt.%, by weight of the composition.

In one embodiment of the invention, the inventive compositions also include an alpha-hydroxy acid.

Hydroxyacids enhance proliferation and increase ceramide biosynthesis in keratinocytes, increase epidermal thickness, and increase desquamation of normal skin resulting in smoother, -younger looking skin.

The hydroxy acid can be chosen from alpha-hydroxy acids, beta-hydroxyacids (e.g. salicylic acid), other hydroxycarboxylic acids (e.g., dihydroxycarboxylic acid,

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hydroxy-dicarboxylic, hydroxytricarboxylic) and mixtures thereof or combination of their stereoisomers (DL, D or L).

Most preferred inventive compositions containing  
5 resveratrol anti-irritant include glycolic acid and/or lactic acid because these ingredients have been found to have potential to cause irritation yet they were found to be particularly efficacious at delivering cosmetic benefits.

10 Preferably the hydroxy acid is chosen from alpha-hydroxy acids having the general structure (1)



15 where M is H or a saturated or an unsaturated, straight or branched hydrocarbon chain containing from 1 to 27 carbon atoms.

Even more preferably the hydroxy acid is chosen from  
20 lactic acid, 2-hydroxyoctanoic acid, hydroxylauric acid, glycolic acid, and mixtures thereof. When stereo isomers exist, L-isomer is most preferred.

A particular advantage of the inventive compositions is  
25 that higher amounts of hydroxy acids may be employed without causing skin irritation. Preferably the amount of the hydroxy acid component present in the composition according to the invention is from 0.01 to 20%, more preferably from 2 to 12% and most preferably from 4 to 12% by weight.

30

It is to be understood that depending on the pH of the composition, the hydroxy acid may be present as a salt, e.g. ammonium or potassium or sodium salt.

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Although the inventive compositions may have any pH in the general range of 2.5 to 10, the inventive compositions are particularly useful when they are at an acidic pH (especially if they contain a hydroxy acid), preferably 3-5 and most preferably at a pH of 3-4, because such compositions are particularly irritating.

#### Optional Skin Benefit Materials and Cosmetic Adjuncts

An oil or oily material may be present, together with an emulsifier to provide either a water-in-oil emulsion or an oil-in-water emulsion, depending largely on the average hydrophilic-lipophilic balance (HLB) of the emulsifier employed.

The inventive compositions preferably include sunscreens. Sunscreens include those materials commonly employed to block ultraviolet light. Illustrative compounds are the derivatives of PABA, cinnamate and salicylate. For example, octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone (also known as oxybenzone) can be used. Octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available under the trademarks, Parsol MCX and Benzophenone-3, respectively. The exact amount of sunscreen employed in the emulsions can vary depending upon the degree of protection desired from the sun's UV radiation.

Emollients are often incorporated into cosmetic compositions of the present invention. Levels of such emollients may range from 0.5% to 50%, preferably between 5% and 30% by weight of the total composition. Emollients may be classified under such general chemical categories as esters, fatty acids and alcohols, polyols and hydrocarbons.

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Esters may be mono- or di-esters. Acceptable examples of fatty di-esters include dibutyl adipate, diethyl sebacate, diisopropyl dimerate, and dioctyl succinate. Acceptable branched chain fatty esters include 2-ethyl-hexyl  
5 myristate, isopropyl stearate and isostearyl palmitate. Acceptable tribasic acid esters include triisopropyl trilinoleate and trilauryl citrate. Acceptable straight chain fatty esters include lauryl palmitate, myristyl lactate, and stearyl oleate. Preferred esters include coco-  
10 caprylate/caprate (a blend of coco-caprylate and coco-caprate), propylene glycol myristyl ether acetate, diisopropyl adipate and cetyl octanoate.

Suitable fatty alcohols and acids include those  
15 compounds having from 10 to 20 carbon atoms. Especially preferred are such compounds such as cetyl, myristyl, palmitic and stearyl alcohols and acids.

Among the polyols which may serve as emollients are  
20 linear and branched chain alkyl polyhydroxyl compounds. For example, propylene glycol, sorbitol and glycerin are preferred. Also useful may be polymeric polyols such as poly-propylene glycol and polyethylene glycol. Butylene and propylene glycol are also especially preferred as  
25 penetration enhancers.

Exemplary hydrocarbons which may serve as emollients are those having hydrocarbon chains anywhere from 12 to 30 carbon atoms. Specific examples include mineral oil,  
30 petroleum jelly, squalene and isoparaffins.

Another category of functional ingredients within the cosmetic compositions of the present invention are thickeners. A thickener will usually be present in amounts  
35 anywhere from 0.1 to 20% by weight, preferably from about

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0.5% to 10% by weight of the composition. Exemplary thickeners are cross-linked polyacrylate materials available under the trademark Carbopol from the B.F. Goodrich Company. Gums may be employed such as xanthan, carrageenan, gelatin, karaya, pectin and locust beans gum. Under certain circumstances the thickening function may be accomplished by a material also serving as a silicone or emollient. For instance, silicone gums in excess of 10 centistokes and esters such as glycerol stearate have dual functionality.

10

Powders may be incorporated into the cosmetic composition of the invention. These powders include chalk, talc, kaolin, starch, smectite clays, chemically modified magnesium aluminum silicate, organically modified montmorillonite clay, hydrated aluminum silicate, fumed silica, aluminum starch octenyl succinate and mixtures thereof.

15

Other adjunct minor components may also be incorporated into the cosmetic compositions. These ingredients may include coloring agents, opacifiers and perfumes. Amounts of these other adjunct minor components may range anywhere from 0.001% up to 20% by weight of the composition.

20

## 25 Use of the Composition

The composition according to the invention is intended primarily as a product for topical application to human skin, especially as an agent for conditioning, moisturizing and smoothening the skin, and preventing or reducing the appearance of lined, wrinkled or aged skin.

30

In use, a small quantity of the composition, for example from 1 to 100ml, is applied to exposed areas of the skin, from a suitable container or applicator and, if

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necessary, it is then spread over and/or rubbed into the skin using the hand or fingers or a suitable device.

#### Product Form and Packaging

5

The topical skin treatment composition of the invention can be formulated as a lotion, a cream or a gel. The composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For  
10 example, a lotion or cream can be packaged in a bottle or a roll-ball applicator, or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container,  
15 such as a tube or a lidded jar. The composition may also be included in capsules such as those described in U.S. Patent 5,063,507, incorporated by reference herein. The invention accordingly also provides a closed container containing a cosmetically acceptable composition as herein defined.

20

The following specific examples further illustrate the invention, but the invention is not limited thereto. In all examples, resveratrol was obtained from Sigma. Student t-test was used to calculate all p-values.

25

#### EXAMPLE 1

This example illustrates that resveratrol is a phytoestrogen.

30

The following test was employed to determine whether resveratrol has an estrogen-like activity:

The ZR75 cell line is a ductal breast carcinoma cell  
35 line, originally isolated from malignant mammary epithelium

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of a sixty-three year old Caucasian female (Engel et al., Human breast carcinoma cells in continuous culture: A review., Cancer Res., 38: 4327-4339, 1978). This cell line contains receptors for estrogen, progesterone and other steroid hormones, but responds through an increase in proliferation only to estrogen. The cell line contains high affinity estrogen-specific receptors. Therefore, this cell line is used for testing estrogen-like activity (Markiewicz et al., In vitro bioassays of non-steroidal phytoestrogens, J. Steroid Biochem. Molec. Biol., 45: 399-405, 1993).

#### Methodology Used for Determining the Rate of DNA Synthesis in Cells:

The incorporation of <sup>3</sup>H-thymidine by cultured cells was used as an assay of cell proliferation (both for ZR75 cells and for keratinocytes). Thymidine is one of four deoxynucleosides which are the monomeric units of DNA. Prior to cell division of a somatic cell, the complete genome of the cell undergoing cell division is replicated. This involves large scale DNA synthesis by the cell and enables both daughter cells to receive identical copies of the genetic material. When <sup>3</sup>H-thymidine is included in the culture media of cells which are synthesizing DNA in preparation for cell division then the labeled thymidine is incorporated into the newly synthesized DNA. The extent of incorporation of <sup>3</sup>H-thymidine into a population of cells is proportional to the rate of DNA synthesis by this population of cells and therefore an indication of their cellular proliferation.

ZR75 cells (from American Type Culture Collection, Rockville, Maryland) were grown in RPMI1640 media (from Gibco Life Technologies) with 10% fetal bovine serum (FBS), 100 units penicillin per ml and 100 units of streptomycin

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per ml. All incubations were performed at 37°C in 5% CO<sub>2</sub>. The media did not contain Phenol Red (a weak estrogen mimetic). The cells were seeded at a density of one million per 75 cm<sup>2</sup> flask. For the experiment, the cells were seeded  
5 in 24 well plates at 100,000 cells per ml per well.

After growing for 24 hours, the media was removed, the cells were washed with PBS (phosphate buffered saline, 0.01 M sodium phosphate, 0.138 M sodium chloride, 0.0027 M  
10 potassium chloride, pH 7.4) and 1 ml of RPMI 1640 without serum (but with streptomycin and penicillin) was added. Stock solutions of resveratrol in dimethyl sulfoxide (DMSO) and estradiol in water were prepared. Various  
15 concentrations of resveratrol and estradiol, as indicated in Table 1, were then dosed directly into each well. After another 24 hours, one µCi of [methyl-3H] thymidine was added to each well. The media was removed after 24 hours. The  
cells were washed once in PBS, the PBS was removed completely and the cells were left on ice to incubate with 1  
20 ml per well of 10% TCA (trichloroacetic acid) for 30 minutes. The plates were washed 3 times with 5% TCA to remove all traces of thymidine which wasn't incorporated into the cells. 500 µl of 0.1M sodium hydroxide was added to each well and the plates were incubated at room  
25 temperature for at least 30 minutes. 250 µl of each sample was transferred to scintillation vials and after adding 5 mL of counting fluid, the vials were counted for 5 minutes each on a setting for tritium. Data from quadruplicate wells  
were calculated as % thymidine incorporation into DNA  
30 compared to that of control wells which did not receive any resveratrol or estradiol. Values were expressed as mean of quadruplicate wells +/- standard deviation.

The results that were obtained are summarized in Table 1



TABLE 1

Compound ( $\mu$ M)	EXPT 1 DNA synthesis. (% of Control)	EXPT 1 p value	EXPT 2 DNA synthesis (% of Control)	EXPT 2 p value
Control (water)	100 $\pm$ 20.4	--	100 $\pm$ 1.9	--
Estradiol (1 nM)	220 $\pm$ 11	0.00059	133.4 $\pm$ 29	0.062
10 nM	210 $\pm$ 9.7	0.00079	152 $\pm$ 17.7	0.0011
100 nM	205 $\pm$ 15.6	0.0016	156 $\pm$ 11.7	0.00008
1000 nM	190 $\pm$ 24.5	0.0068	142 $\pm$ 18.3	0.0039
Control (DMSO)	100 $\pm$ 7.0	--	100 $\pm$ .08	--
Resveratrol (0.5 $\mu$ M)	69.7 $\pm$ 10.0		56.8 $\pm$ 4.2	
1 $\mu$ M	58.8 $\pm$ 9.0		60.5 $\pm$ 3.6	
5 $\mu$ M			158.8 $\pm$ 4.1	0.000008
10 $\mu$ M	207.8 $\pm$ 32.5	0.0026	163 $\pm$ 13.7	0.00029
15 $\mu$ M			119 $\pm$ 2.5	0.00009
20 $\mu$ M	134.8 $\pm$ 21.4	0.612	68 $\pm$ 3.9	
40 $\mu$ M	60.8 $\pm$ 26.4			
50 $\mu$ M	4.1 $\pm$ 1.4			

The control  $^3$ H thymidine incorporation value for  
 5 experiment 1 was 71513 cpm and the control for experiment 2  
 was 114958 cpm.

The results in Table 1 demonstrate that estradiol, a  
 known estrogen, stimulated proliferation of ZR 75 cells, as

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expected. Resveratrol increased proliferation of ZR 75 cells at a concentration from 5 to 20  $\mu$ M.

## EXAMPLE 2

5

This example demonstrates that resveratrol inhibits proliferation of keratinocytes.

1. Normal human keratinocytes isolated from neonatal  
10 foreskins by trypsin treatment were grown in Dulbecco's modified Eagle's medium (DME)/5% fetal calf serum in the presence of mitomycin C treated 3T3 mouse fibroblasts for establishing dividing keratinocyte colonies. Keratinocytes were grown under the above condition until  
15 their third passage.
2. For the experiments, third passage keratinocytes were plated into a serum-free keratinocyte growth medium (KGM; obtained from Clonetics, San Diego, California)  
20 containing 0.09 mM calcium. About 30,000 cells were plated into each well of 6 well cell culture plates and grown for 5 days, until the cells reach about 40% confluence.
- 25 3. Medium was changed to fresh medium (KBM; obtained from Clonetics) and resveratrol at various concentrations as indicated in Table 2 was added to the medium from a DMSO (Dimethylsulfoxide) stock solution. The final DMSO concentration in the cultures was 0.1%. Control cultures  
30 received no resveratrol but were dosed with 0.1% DMSO. Each concentration was tested in three separate wells. After four hours, 1  $\mu$ Ci of  $^3$ H-thymidine (Amersham Corp., Sp activity 40 Ci/mmol) was added to 1 ml of medium in each well. The cells were incubated for 2 hours. The

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amount of  $^3\text{H}$ -thymidine associated with the cellular DNA of keratinocytes was assessed as described below.

4. The medium was aspirated, and the wells washed with 1 ml  
5 PBS. The DNA and proteins of the cells in the plate were  
then precipitated by adding 1 ml of ice-cold 10% TCA.  
The plates were left on ice for 30 minutes to complete  
the precipitation process. TCA was then aspirated and  
each well was then washed 4 times with 5% TCA. The cells  
10 in the wells were dissolved in 0.5 ml of 0.1N sodium  
hydroxide. 200 $\mu\text{l}$  was then transferred to a scintillation  
vial for assessing thymidine incorporation and 25  $\mu\text{l}$  was  
used for a protein assay using BCA protein assay reagent  
as described below. 5 ml of a scintillation fluid  
15 (Scintiverse) was added to the rest of the solution in  
the vial, and the vials were counted in a scintillation  
counter to determine the amount of radioactivity in each  
vial.

20 BCA (Bicinchoninic Acid) - Protein Assay

- 25 $\mu\text{l}$  of cell suspension were placed in a 96 well plate.  
Standards of BSA (bovine serum albumin) in 0.1N Sodium  
Hydroxide were also pipetted in triplicate in the same 96  
25 well plate. Pierce BCA protein assay reagent was added  
(200 $\mu\text{l}$ /well) and plate was incubated for 2 hours at room  
temperature. Absorbance was read at a wavelength of 570nm  
on a Dynatech MR7000 plate reader.

- 30 The DNA synthesis rate was then calculated as cpm  $^3\text{H}$ -thymidine  
incorporated into total cellular DNA/ $\mu\text{g}$  of cell protein for  
each individual well. Mean and standard deviation for each  
group were also calculated. These numbers were also expressed  
as percent of control wells. Each data point is expressed as  
35 mean of triplicate wells  $\pm$  standard deviation.

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The results that were obtained are summarized in Table 2.

p values of less than 0.5 were considered to indicate statistical significance.

5

TABLE 2

Resveratrol ( $\mu$ M)	DNA synthesis (cpm/ $\mu$ g protein)	% inhibition	P value
Experiment 1			
0	29.52 $\pm$ 4.44	0	--
50	0.62 $\pm$ 0.09	98	0.012
Experiment 2			
0	17.17 $\pm$ 1.21	0	--
0.78	11.57 $\pm$ 1.7	33	0.053
1.56	8.89 $\pm$ 1.12	48	0.016
3.12	5.11 $\pm$ 0.27	70	0.0074
6.25	2.45 $\pm$ 0.56	86	0.0053
12.5	1.07 $\pm$ 0.11	94	0.003
25	0.58 $\pm$ 0.05	97	0.0029
50	0.44 $\pm$ 0.12	97	0.0028

As can be seen from the results in Table 2, concentrations as low as 1.56  $\mu$ M resveratrol decreased DNA synthesis of keratinocytes significantly. 1.56  $\mu$ M resveratrol reduced keratinocyte proliferation by as much as 50%. In both experiments, 50  $\mu$ M resveratrol inhibited DNA synthesis completely.

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## EXAMPLE 3

Example 2 was repeated at various additional concentrations of resveratrol. The results that were obtained are summarized in Table 3.

TABLE 3

EFFECT OF RESVERATROL AT LOW CONCENTRATIONS  
ON THYMIDINE UPTAKE IN KERATINOCYTES

Resveratrol concentration	DNA synthesis (cpm/ $\mu$ g protein)	% inhibition	P value	Statistically Significant (at p less than 0.5)
0.0 $\mu$ M	63.4 $\pm$ 5.2	---	---	---
0.1 $\mu$ M	60.4 $\pm$ 4.1	5	0.502	NO
0.2 $\mu$ M	56.3 $\pm$ 3.0	12	0.079	NO
0.3 $\mu$ M	58.8 $\pm$ 0.4	7	0.254	NO
0.4 $\mu$ M	54.2 $\pm$ 1.2	14	0.051	NO
0.6 $\mu$ M	59.3 $\pm$ 5.5	6	0.310	NO
0.8 $\mu$ M	44.4 $\pm$ 2.6	30	0.00013	YES
1.0 $\mu$ M	37.7 $\pm$ 4.0	40	<0.0001	YES
2.0 $\mu$ M	25.7 $\pm$ 2.4	59	<0.0001	YES

It can be seen from the results in Table 3 that resveratrol was not effective at reducing keratinocyte proliferation at concentrations lower than 0.8  $\mu$ M (or 0.000018 wt. %), including a very low concentration of 0.33  $\mu$ M which would be the maximum concentration present in 0.5% grape extract disclosed by JP 6336421.

## EXAMPLE 4

This example demonstrates that resveratrol induces differentiation of keratinocytes:

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## Methodology for transglutaminase measurement:

During the process of terminal differentiation in the epidermis, a 15nm thick layer of protein, known as the cornified envelope (CE) is formed on the inner surface of the cell periphery. The CE is composed of numerous distinct proteins which have been cross-linked together by the formation of N'-((-glutamyl) lysine isodipeptide bonds catalyzed by the action of at least two different transglutaminases expressed in the epidermis. Transglutaminase (TG-1) is expressed in abundance in the differentiated layers of the epidermis, especially the granular layer, but is absent in the undifferentiated basal epidermis. Thus, TG-1 is a useful marker of epidermal keratinocyte differentiation with high TG-1 levels indicating a more differentiated state. An ELISA based TG-1 assay, using a TG-1 antibody, was used to assess the state of differentiation of the cultured keratinocytes in the examples that follow.

The level of TG-1 was measured as follows. Keratinocytes were obtained as described in Example 2. For the experiment, about 30,000 cells were plated into each well of 6 well plates and grown for five days, until the cells reach about 20-30% confluence. 2ml/well of fresh KGM were added daily with 2µl of 2-50mM resveratrol in DMSO for 3 days. Control wells also received 2µl of DMSO. After 3 days of treatment, cells were washed twice with PBS and placed in freezer for 2 hours. Cells were then thawed for 2 hours. DNA content of the cells were quantitated by using the DNA binding flurophore, bis-benzimidazole (Hoechst 33258) and measuring the specific fluorescence of the DNA-bound flurophore at 450 nm (excitation at 360 nm).

TG-1 levels of the cells in the wells were determined using the TG-1 specific monoclonal antibody (BC1) (first

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antibody) (obtained from Amersham Life Sciences) and using a peroxidase labeled rabbit antimouse IgG fragment (second antibody). The plates were blocked by 5% nonfat milk in TBS (Tris buffered saline, 0.01 M Tris, 0.150 M sodium chloride, pH 8.0) for one hour followed by 2 hour incubation with the first antibody (1:4,000 fold dilution) in 1% milk/TBS at room temperature. After rinsing the plates three times with 1% milk/TBS containing 0.05% Tween 20, the plates were incubated with 1:4000 dilution of the second antibody at room temperature for two hours. The plates were rinsed three times with 1% milk/TBS/Tween and three times with TBS. Color was developed by incubation with o-phenylene diamine and hydrogen peroxide. The absorbance was read at 492 nm on a Ultrospec 3000 spectrophotometer (Pharmacia Biotech) and TG-1 levels were calculated as Abs/DNA fluorescence. The mean  $\pm$  standard deviation of at least 3 separate wells were used for calculation and statistical analysis of the data. Values were expressed as absorbance for TG-1 per arbitrary unit of DNA fluorescence of triplicate wells  $\pm$  standard deviation. Results were also expressed as % of control.

The results that were obtained are summarized in Table 4.

TABLE 4

Resveratrol ( $\mu$ M)	TG-1 levels/ $\mu$ g DNA	% of control	p value
EXPERIMENT 1			
0 $\mu$ M	6.74 $\pm$ 0.79	---	---
10 $\mu$ M	8.49 $\pm$ 1.77	126	0.1195
25 $\mu$ M	10.92 $\pm$ 2.29	162	0.0008
50 $\mu$ M	22.2 $\pm$ 1.50	329	0.0001
EXPERIMENT 2			
0 $\mu$ M	1.91 $\pm$ 0.03	---	---
2 $\mu$ M	3.42 $\pm$ 0.42	179	<0.0001
5 $\mu$ M	3.51 $\pm$ 0.41	184	<0.0001
10 $\mu$ M	3.61 $\pm$ 0.17	189	<0.0001
25 $\mu$ M	4.69 $\pm$ 0.21	246	<0.0001
50 $\mu$ M	4.65 $\pm$ 0.46	243	<0.0001

10  $\mu$ M resveratrol was not significantly different from control in Experiment 1 due to normal experimental variations in biological systems, but all other concentrations significantly increased transglutaminase expression of keratinocytes, thus proving that resveratrol increases keratinocyte differentiation. In Experiment 2 all concentrations of resveratrol of 2 $\mu$ M and higher significantly increased keratinocyte differentiation.

## EXAMPLE 5

This example demonstrates that resveratrol alleviates skin inflammation that may be caused by alpha-hydroxy acids.

Resveratrol is a known cyclo-oxygenase inhibitor. Inhibition of cyclooxygenase reduces the conversion of arachidonic acid to pro-inflammatory substances such as



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prostaglandins, including PGE2. While inhibition of cyclooxygenase would be expected to reduce inflammation, not all cyclooxygenase inhibitors reduce irritation potentially associated with a cosmetic ingredient such as alpha hydroxy acids.

#### Example 5A

#### Irritation Test Method:

10

Four Exposure Patch Test: The objective was to compare the level of irritation produced by various test materials after repeated patch applications. The test materials were held in contact with the skin under occlusive conditions.

15 The outer upper arm of the panelist was designated as the area of application. Bandage type dressing (Scanpor tape) was used to hold the patches (25 mm Hill Top Chamber fitted with 18 mm diameter disc of Webril padding) into place. Both upper arms of the panelist were used. Patches were

20 applied in a balanced random order.

Patches were applied at 9:00 o'clock Monday morning and removed at 9:00 o'clock Tuesday morning (24 hour exposure). A new set of patches was applied at 3:00 o'clock Tuesday

25 afternoon and removed Wednesday morning at 9:00 o'clock (18 hour exposure). A third set of patches was applied at 3:00 o'clock Wednesday afternoon and removed Thursday morning at 9:00 o'clock (18 hour exposure). A final set of patches was applied at 3:00 o'clock Thursday afternoon and removed

30 Friday morning at 9:00 o'clock (18 hour exposure).

Each time the patches were removed, the sites were rinsed with warm water and patted dry. The test sites were then marked with a surgical skin marking pen to ensure

35 location for grading and subsequent patch applications.

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Test sites were evaluated at 3:00 p.m. on Tuesday, Wednesday, Thursday, and Friday of the study, prior to re-patching.

5 Skin irritation such as moderate redness, dryness, and/or itching of the test site is expected. Swelling of the test sites was possible. If any test site had moderate redness or any swelling at any evaluation, that particular test site was not repatched.

10

The test sites on each arm were visually ranked by two trained examiners under consistent lighting. The test sites were ranked in order of severity. The examiner ranking responses at the first evaluation period continued ranking  
15 the sites each day throughout the study.

In ranking the reactions, the site with the most severe response was given the lowest score. The site with the second most severe response was given the second lowest  
20 score, etc. There was no forced ranking. If two or more sites had no response or the same response (no difference between sites), an average of the ranks was assigned. If a site had been discontinued, due to degree of irritation, the site retained the rank it received at the time dosing was  
25 discontinued.

#### Statistical Analysis:

The ranking results from the patch treatments were  
30 statistically compared by nonparametric statistical methods. The test materials containing the anti-irritants were compared to the corresponding control containing only hydroxy acid, using Friedman's Rank Sum at each evaluation point with the panelist acting as a block (i.e., each  
35 panelist was tested with each test treatment). A p-value of

- 25 -

less than 0.10 was considered to indicate statistical significance.

5 Compositions containing ingredients as indicated in Table 5A, were tested using the Irritation Test Method. Twenty (20) subjects were tested. The results that were obtained are summarized in Table 5A. The higher the sum of ranks, the less is the irritation.

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## BASE FORMULA

FULL CHEMICAL NAME OR CFTA NAME	TRADE NAME AND % ACTIVE	WT. %
water, DI		46.54
disodium EDTA	Sequesterene Na2	0.05
magnesium aluminum silicate	Veegum Ultra	0.6
methyl paraben	Methyl Paraben	0.15
simethicone	DC Antifoam Emulsion	0.01
butylene glycol 1,3	Butylene Glycol 1,3	3.0
hydroxyethylcellulose	Natrosol 250HHR	0.5
glycerine, USP	Glycerine USP	2.0
xanthan gum	Keltrol 1000	0.2
triethanolamine	Triethanolamine 99 (%)	1.2
stearic acid	Pristerene 4911	3.0
propyl paraben NF	Propylparaben NF	0.1
glyceryl hydrostearate	Naturechem GMHS	1.5
stearyl alcohol	Lanette 18DEO	1.5
isostearyl palmitate	Protachem ISP	6.0
C12-15 alcohols octanoate	Hetester FAO	3.0
dimethicone	Silicone Fluid 200 (50cts)	1.0
cholesterol NF	Cholesterol NF	0.5
sorbitan stearate	Sorbitan Stearate	1.0
butylated hydroxytoluene	Embanox BHT	0.05
tocopheryl acetate	Vitamin E Acetate	0.1
PEG-100 stearate	MYRJ 59	2.0
sodium stearyl lactylate	Pationic SSL	0.5
retinyl palmitate	Vit. A Palmitate 84%	0.06
hydroxy caprylic acid	Hydroxy caprylic acid	0.1
water, DI		q.s. to 99.80
alpha-bisabolol	Alpha-bisabolol	0.2
pH		7-8

Table 5A

COMPOSITION	INGREDIENTS	SUM OF RANKS (Day 1)	SUM OF RANKS (Day 4)
1	Base Formula	65.5	79.5
2	Base Formula + 8% Glycolic acid	63	72
3	Composition #2 + 0.1% resveratrol	85 <sup>a</sup>	66

a: significantly less irritating than composition 2.

5

It can be seen from the results in Table 5A that resveratrol (Composition 3) significantly reduced the irritation induced by composition #2 (containing 8% glycolic acid) on Day 1, after the initial exposure to composition #

10

#### Comparative Example 5B

Compositions containing ingredients as indicated in Table 5B, were tested using the Irritation Test Method described in Example 5A. Twenty-two (22) subjects were tested. The results that were obtained are summarized in Table 5B. The higher the sum of ranks, the less is the irritation.

20

Table 5B

COMPOSITION	INGREDIENTS	SUM OF RANKS (Day 1)	SUM OF RANKS (Day 4)
1	Base Formula	81	90.5
2	Base Formula + 8% Glycolic acid	75	73.5
4	Composition #2 + 5% Ibuprofen	71.5	65.5

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It can be seen from the results in Table 5B, that ibuprofen, a known anti-inflammatory ingredient (composition #4) did not reduce the irritation of the Formula which contains 8% glycolic acid (composition #2).

5

#### Comparative Example 5C

Compositions containing ingredients as indicated in Table 5C, were tested using the Irritation Test Method, as described in Example 5A. Nineteen (19) subjects were tested. The results that were obtained are summarized in Table 5C. The higher the sum of ranks ranks, the less is the irritation.

Table 5C

15

COMPOSITION	INGREDIENTS	SUM OF RANKS (Day 1)	SUM OF RANKS (Day 4)
2	Base Formula + 8% Glycolic acid	70	62.5
5	Composition #2 + 1% Indomethacin	53.5	52.5

It can be seen from the results in Table 5C, that indomethacin, a known cyclo-oxygenase inhibitor and anti-inflammatory ingredient (composition #5) did not reduce the irritation of the Formula which contains 8% glycolic acid (composition #2).

20

Examples 6-11 illustrate skin care compositions according to the present invention. The compositions can be processed in conventional manner. They are suitable for cosmetic use. In particular, the compositions are suitable for application to wrinkled, lined, rough, dry, flaky, aged and/or UV-damaged skin to improve the appearance and the feel thereof as well as for application to healthy skin to prevent or retard deterioration thereof. The composition are also particularly

25

30

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suitable to reduce the irritation, sting, or inflammation that may be associated with the use of alpha-hydroxy acids.

## EXAMPLE 6

5

This example illustrates a high internal phase water-in-oil emulsion incorporating the inventive composition.

	% w/w
RESVERATROL	0.5
1,3-dimethyl-2-imidazolidinone	0.2
Brij 92*	5
Bentone 38	0.5
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.3
Butylated hydroxy toluene	0.01
Perfume	qs
Water	to 100

10

\* Brij 92 is polyoxyethylene (2) oleyl ether

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## EXAMPLE 7

This example illustrates an oil-in-water cream incorporating the inventive composition.

5

	% w/w
RESVERATROL	2
Glycolic Acid	8
Mineral oil	4
1,3-dimethyl-2-imidazolidinone	1
Brij 56*	4
Alfol 16RD*	4
Triethanolamine	0.75
Butane-1,3-diol	3
Xanthan gum	0.3
Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100

\* Brij 56 is cetyl alcohol POE (10)

Alfol 16RD is cetyl alcohol

10



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## EXAMPLE 8

This example illustrates an alcoholic lotion incorporating the composition according to the invention.

5

	% w/w
RESVERATROL	5
1,3-dimethyl-2-imidazolidinone	0.1
Ethanol	40
Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100

## EXAMPLE 9

10 This example illustrates another alcoholic lotion containing the inventive composition.

	% w/w
RESVERATROL	10
1,3-dimethyl-2-imidazolidinone	0.01
Ethanol	40
Antioxidant	0.1
Perfume	qs
Water	to 100

## EXAMPLE 10

This example illustrates a suncare cream incorporating the composition of the invention:

5

	% w/w
RESVERATROL	2
1,3-dimethyl-2-imidazolidinone	0.2
Silicone oil 200 cts	7.5
Glycerylmonostearate	3
Cetosteryl alcohol	1.6
Polyoxyethylene-(20)-cetyl alcohol	1.4
Xanthan gum	0.5
Parsol 1789	1.5
Octyl methoxycinnate (PARSOL MCX)	7
Perfume	qs
Color	qs
Water	to 100

## EXAMPLE 11

This example illustrates a non-aqueous skin care composition incorporating the inventive combination.

5

	% w/w
RESVERATROL	5
1,3-dimethyl-2-imidazolidinone	1
Silicone gum SE-30 <sup>1</sup>	10
Silicone fluid 345 <sup>2</sup>	20
Silicone fluid 344 <sup>3</sup>	50.26
Squalene	10
Linoleic acid	0.01
Cholesterol	0.03
2-hydroxy-n-octanoic acid	0.7
Vitamin E linoleate	0.5
Herbal oil	0.5
Ethanol	2

<sup>1</sup> A dimethyl silicone polymer having a molecular weight of at least 50,000 and a viscosity of at least 10,000 centistokes at 25°C, available from GEC

10 <sup>2</sup> Dimethyl siloxane cyclic pentamer, available from Dow Corning Corp.

<sup>3</sup> Dimethyl siloxane tetramer, available from Dow Corning Corp.

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CLAIMS

1. A skin care composition comprising resveratrol in an amount of from 0.00002 to 10 wt.% and a cosmetically acceptable vehicle.  
5
2. A cosmetic method of delivering a phytoestrogen to the human skin, the method comprising applying to the skin the composition comprising resveratrol in an amount of  
10 from 0.00002 to 10 wt.% and a cosmetically acceptable vehicle.
3. A cosmetic method of improving the appearance of wrinkled, lined, dry, flaky, aged or photodamaged skin and improving skin thickness, elasticity, flexibility and plumpness, the method comprising applying to the skin the composition of claim 1.  
15
4. A cosmetic method of enhancing keratinocyte differentiation in human skin, the method comprising applying to the skin the composition of claim 1.  
20
5. A cosmetic method of inhibiting keratinocyte proliferation in human skin, the method comprising  
25 applying to the skin the composition of claim 1.
6. A skin care composition comprising
  - (a) resveratrol in an amount of from 0.00002 to  
30 10 wt.%;
  - (b) hydroxy acid in an amount of from 0.01% to 20 %; and
  - (c) a cosmetically acceptable vehicle.
7. The composition of claim 7 wherein the amount of the  
35 hydroxy acid is from 2% to 12%.



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/EP98/04223 <b>(22) International Filing Date:</b> 7 July 1998 (07.07.98) <b>(30) Priority Data:</b> 08/900,795 25 July 1997 (25.07.97) US <b>(71) Applicant (for AU BB CA CY GB GH GM IE IL KE LC LK LS MN MW NZ SD SG SL SZ TT UG ZW only):</b> UNILEVER PLC [GB/GB]; Unilever, Blackfriars, London EC4P 4BQ (GB). <b>(71) Applicant (for all designated States except AU BB CA CY GB GH GM IE IL KE LC LK LS MN MW NZ SD SG SL SZ TT UG ZW):</b> UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL). <b>(72) Inventors:</b> CARSON, Robert, George; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). PATEL, Krupa; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). CARLOMUSTO, Marieann; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). BOSKO, Carol, Annette; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US). PILLAI, Sreekumar; Unilever Research U.S. Inc., 45 River Road, Edgewater, NJ 07020 (US).		<b>(74) Agent:</b> ROTS, M., J., F.; Unilever plc, Patent Division, Colworth House, Sharnbrook, Bedford MK44 1LQ (GB). <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <b>(88) Date of publication of the international search report:</b> 8 April 1999 (08.04.99)
<b>(54) Title:</b> COSMETIC COMPOSITIONS <b>(57) Abstract</b> <p>Resveratrol, a component of a variety of common edible plants, including peanuts and red grapes, is a phytoestrogen. Resveratrol inhibits proliferation of skin epidermal cells (keratinocytes) and stimulates their differentiation. Resveratrol was also found to alleviate skin irritation that may be caused by alpha-hydroxy acids. Resveratrol is useful in improving the appearance of wrinkled, lined, dry, flaky, aged or photodamaged skin and improving skin thickness, elasticity, flexibility, radiance, glow and plumpness.</p>		

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# INTERNATIONAL SEARCH REPORT

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## B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	PATENT ABSTRACTS OF JAPAN vol. 098, no. 006, 30 April 1998 & JP 10 045566 A (YUSHIRO CHEM IND CO LTD; MITSUBA BOEKI KK), 17 February 1998 see abstract	1-3
P, X	PATENT ABSTRACTS OF JAPAN vol. 098, no. 004, 31 March 1998 & JP 09 328410 A (YUSHIRO CHEM IND CO LTD; MITSUBA BOEKI KK), 22 December 1997 see abstract	1-3



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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

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Couckuyt, P

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X	CHEMICAL ABSTRACTS, vol. 111, no. 14, 2 October 1989 Columbus, Ohio, US; abstract no. 120645, XP002090494 & JP 01 038009 A (POLA CHEMICAL INDUSTRIES INC.) 8 February 1989 see abstract ---	1-3
X	STN FILE SUPPLIER EMBASE ELSEVIER SCI. B.V., XP002090493 AN:95287549 & BOMBARDELLI ET AL.: "VITIS VINIFERA L." FITOTERAPIA, vol. 66, no. 4, 1995, pages 291-317, ITALY see abstract -----	1